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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/940,692	09/30/97	VALLE F	GCL266-2

HM22/0703

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EXAMINER	
PROUTY,R	
ART UNIT	**PAPER NUMBER**
1652	24

DATE MAILED: 07/03/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applic(s)
08/940,692	Valle et al.
Examiner	**Group Art Unit**
Rebecca Prouty	1652

☒ Responsive to communication(s) filed on *Apr 10, 2000*

☐ This action is **FINAL.**

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claim

☒ Claim(s) *23-31 and 33-46* is/are pending in the applicat

Of the above, claim(s) _____ is/are withdrawn from consideration

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *23-31 and 33-46* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☒ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

The request filed on 4-10-00 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 08/940,962 is acceptable and a CPA has been established. An action on the CPA follows.

Claims 1-22 and 32 have been canceled. Claims 23-31, 33-39 and newly presented claims 40-46 are still at issue and are present for examination.

Applicants' arguments filed on 10-13-99, paper No. 19, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim 27 is objected to because of the following informalities: the word "into" in line 2 should be "to". Appropriate correction is required.

Claims 23-26, 40, 41, and 43-44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 23 (upon which Claims 24-26, 40 and 41 depend) is confusing in the recitation on "host cell comprising a metabolic pathway" as metabolic pathways are not components of cells.

Claims 43 and 44 are confusing in the recitation of "The mutant cell of Claim 42" as Claim 42 recites a method not a mutant cell. As it is unclear what was intended to be claimed here, these claims have not been further examined.

Claims 38, 39 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of obtaining a $Pts^-/glucose^+$, galactose permease requiring mutant cells by mutating the host cell to inactivate the phosphotransferase system and selecting for those cells with a high growth rate on glucose, does not reasonably provide enablement for methods of obtaining a $Pts^-/glucose^+$, galactose permease requiring mutant cells by mutating the host cell in any fashion and selecting for those cells with a high growth rate on glucose. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

These claims are drawn to methods of obtaining a $Pts^-/glucose^+$, galactose permease requiring mutant cell by mutating the host cell and selecting for those cells with a high growth rate on glucose. However, the claims lack any step which selects for cells with a Pts^- phenotype. If one merely subjects cells to a mutagenesis procedure, the mutations produced will occur

throughout the genome of the organism with only a very small percentage of the cells having mutations which will effect the phosphotransferase system. The remaining steps of the claimed method do not provide any means of selecting for those cells which lack such a system. As such it would require undue experimentation to use the claimed method to obtain Pts^- /$glucose^+$, galactose permease requiring mutant cells without an additional selection step for those cells having a Pts^- phenotype.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 23, 27, 28, 38, 41, 45, and 46 are rejected under 35 U.S.C. § 102(b) as being anticipated by Saier et al.

Saier et al. teach methods of selecting a Pts^-/$glucose^+$ cell comprising deleting the PTS genes (*ptsH* and *ptsI*), culturing the mutant cell using glucose as the sole available carbon source and selecting cells with a fast growth rate on glucose.

Applicants argue that Claims 23, 27, 28, 38, 41, 45, and 46 are novel as Saier et al. did not select for fast growing cells

having a growth rate of at least about 0.4/hr. This is not persuasive because the cells isolated by Saier et al. are fast growing as claimed. Table 1 shows that the PTS^-/glu^+ mutants have a generation time of 2 hrs. Applicants argue that a doubling time of 2 hours does not meet the limitation of a specific growth rate of at least about 0.4 hr^{-1}. This is not persuasive because a doubling time of 2 hrs as disclosed by Saier et al. is equivalent to a specific growth rate of 0.35 hr^{-1}. This clearly falls within the scope of at least about 0.4 hr^{-1}.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. § 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant

is advised of the obligation under 37 C.F.R. § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. § 102(f) or (g) prior art under 35 U.S.C. § 103.

Claims 23-31, 33-38, 41, 42, 45, and 46 are rejected under 35 U.S.C. § 103 as being unpatentable over the combined disclosures of Frost, Holms, Ingraham et al. and Saier et al.

Frost teaches the amplification of carbon flow into the common aromatic pathway by increasing the amount of one of the substrates (E4P) for the first committed step of this pathway (i.e., the DAHP synthetase catalyzed condensation of E4P and PEP) by introduction of the transketolase gene into the host cell. He further teaches the introduction of one or more of the genes of the common aromatic pathway in such cells to further increase the amount of the desired final product.

Holms teaches that PEP within *E. coli* is consumed by several different metabolic pathways (i.e., the PTS system, pyruvate synthesis by pyruvate kinase, and oxaloacetate synthesis by phosphoenolpyruvate carboxylase) and the amount of PEP channeled into each of these pathways. Holms teaches that the PTS system consumes 66% of the PEP produced while only 3% of the PEP pool is channeled into aromatic amino acid synthesis.

Ingraham et al. teach that it would be advantageous to increase the supply of PEP in a cell used for production of a desired product, in particular aromatic amino acid production, by modifying an enteric bacteria such as *E. coli* to use an alternative pathway from the PTS system for glucose uptake such that PEP production is not obligately coupled to glucose transport.

Saier et al. teach methods of selecting a $Pts^-/glucose^+$ cell which uses galactose permease to transport glucose comprising deleting the PTS genes (*ptsH* and *ptsI*), culturing the mutant cell using glucose as the sole available carbon source and selecting cells with a fast growth rate on glucose.

The disclosure of Frost of amplification of carbon flow into the common aromatic pathway by increasing the amount of one of the substrates (E4P) for the first committed step of this pathway would suggest to the ordinary skilled artisan the amplification of the other necessary precursor (i.e., PEP) of this enzymatic step as this would assure that neither substrate for this enzyme would be in limiting supply. One of ordinary skill in the art would recognize that the supply of any precursor used by a cellular pathway could be amplified by either increasing the amount of the precursor synthesized (such as done by Frost for

E4P) or by preventing the depletion of the precursor by other cellular pathways thereby increasing the amount of the precursor available to be used by the desired pathway. The disclosure of Holms that 66% of the cellular PEP is used by the competing PTS pathway would suggest to the ordinary skilled artisan that PEP availability to the common aromatic pathway could be substantially increased by preventing PEP use by the PTS pathway. Furthermore, Ingraham et al. explicitly suggest this as an approach to increasing the level of carbon flow into the common aromatic pathway. The disclosure of Saier et al. shows that it is possible to produce cells which are deleted in the PTS system yet still retain high growth rates on glucose (a carbon source normally transported by the deleted PTS system) by utilizing the galactose permease as a means of glucose transport. Therefore, it would have been obvious to one of ordinary skill in the art to produce a $Pts^-/glucose^+$ mutant of the host cells of Frost which exhibit high levels of carbon flow into the common aromatic pathway as one of ordinary skill in the art would reasonably expect such a mutant cell to divert higher levels of the cellular pool of PEP into the aromatic amino acid biosynthetic pathways and produce further increases in the amount of carbon flow into this pathway. It would have been further obvious to one of ordinary skill in the art to select for such cells with high

growth rates as such cells would be expected to be most useful for producing large amounts of aromatic amino acids. Furthermore, it would have been further obvious to one of ordinary skill in the art to further increase the amount of PEP diverted into this pathway by preventing its use by the other metabolic pathways which Holms teach that it is consumed by. As such it would have been obvious to further mutate the pyruvate kinase and pyruvate carboxylase genes as well.

Applicant's argue that the 103 rejection should be withdrawn as the cited references fail to provide sufficient motivation to increase PEP availability. This is not persuasive because Ingrahm et al. specifically suggest preventing PEP use by the PTS system (while providing another means of glucose transport) as an approach to increasing the carbon flow into the common aromatic pathway. Ingrahm et al. state in column 1:

> "Phosphoenol pyruvate (PEP) is a central intermediate in glucose metabolism, residing at a branch point for the biosynthesis of many compounds of commercial importance. For example, an equimolar amount of PEP is combined with erythrose-4-phosphate to provide the carbon skeleton for aromatic products such as tyrosine, phenylalanine, tryptophan, and some vitamins among other compounds."

and further go on to state:

> "In many bacteria, PEP is also necessary for the transport of glucose into the cell. Glucose is phosphorylated in a concerted process by a multiprotein -membrane-bound complex termed the phosphotransferase

> system (PTS). In this process, PEP serves as the source of a high energy phosphate which is ultimately attached to glucose to yield glucose-6-phosphate and pyruvate. During glycolysis in these organisms, half the PEP produced is obligately consumed to provide energy for glucose uptake. this reduces by 50% the amount of PEP available as a source of carbon skeletons for biosynthesis, severely impacting the efficiency of conversion into many desired commercial products."

Finally in column 3, Ingrahm et al. state:

> "For example, by modifying an enteric bacteria such as *E. coli* to use an alternative pathway for glucose uptake characteristic of *Z. mobilis*, the output of any synthetic product derived from PEP as a precursor could be doubled because glucose transport into cells would not be obligately coupled to PEP."

Therefore, Ingrahm et al. explicitly suggest the uncoupling of glucose transport and PEP utilization as a means of increasing the carbon flow into the common aromatic pathway. While Ingrahm et al. suggest doing this by a different means than that suggested by applicants, Saier et al. clearly teach that other means of providing glucose transport to a PTS^- cell are known in the art. As such it would have been obvious to one of ordinary skill in the art that the same objective explicitly stated in Ingrahm et al. could be provided by the PTS^-/glu^+ cells of Saier et al.

Applicants should note that the above rejections have been withdrawn for those claims limited to cells having a specific growth rate of at least 0.8/hr as Saier et al. fail to teach that

one can obtain PTS^-/glu^+ cells with a growth rate this high. While one of ordinary skill in the art would clearly have been motivated to look for cells with higher growth rates than those obtained by Saier et al., the ordinary skilled artisan would expect that limitations on the ability of the cell to transport glucose might inhibit the rate of growth of the cells on this sugar such that one could not reasonably expect that such cells could be made without a demonstration thereof. As such it would have merely been obvious to try to obtain cells with this claimed higher growth rate.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rebecca Prouty, Ph.D. whose telephone number is (703) 308-4000. The examiner can normally be reached on Monday-Friday from 8:30 to 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy, can be reached at (703) 308-3804. The fax phone number for this Group is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Rebecca Prouty
Primary Examiner
Art Unit 1652